Tracing the Origins of Gain-of-Function Research to Polio (Chow, 1991) and Coronavirus (Rottier, 2000)

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Gain-of-function (GoF) research has been a powerful tool in virology, unlocking insights into viral pathogenicity, transmission, and host range. However, it also raises significant ethical and safety concerns. This essay delves into two seminal studies from the early era of GoF research: Rottier's manipulation of coronavirus host range and Chow's investigation into poliovirus neutralization sites. These pioneering works not only advanced our understanding of viral biology but also shaped the regulatory landscape for GoF research, providing enduring lessons for the scientific community navigating this complex field today.

Keywords: gain-of-function research, Rottier, Chow, coronavirus, poliovirus, host range, viral neutralization sites, NIH pause, bioethics, biosafety, virology, regulation, vaccine development, antiviral strategies, zoonotic diseases.

Note: I allowed GPT-4 to write on its own; however, I see these two papers as seminal in bioweapons development, and the research should have been banned. Please consider the AI bias in writing as of historic importance. - DCY

In the world of microbiology and virology, there exists a set of investigations known as gain-of-function (GoF) research. As the name implies, these studies aim to understand the effects of specific alterations to an organism's genome that would confer an advantage or 'gain' of biological function. Specifically, in the context of virology, these investigations typically focus on modifications that could potentially enhance a virus's ability to spread, adapt to new hosts, or evade the host's immune responses.

Gain-of-function research, while critically informative, poses complex questions due to the inherent risks associated with it. These experiments, after all, involve the deliberate alteration of pathogenic agents, potentially enhancing their virulence or transmissibility. The debates around these questions strike at the core of scientific inquiry, encompassing ethics, risk management, and the broader societal implications of our quest for knowledge.

Two early and influential papers embody the promise and challenges of gain-of-function research: one by Rottier et al., investigating the effects of manipulating a coronavirus's spike protein to alter its host range, and another by Chow et al., delving into the consequences of site-specific mutations in poliovirus neutralization sites. It's important to underscore that these papers were written before the formal recognition of GoF as a distinct field of study and certainly prior to the implementation of contemporary regulations and precautions surrounding such research.

Rottier and Chow's work, conducted in the late 20th and early 21st centuries, respectively, provided groundbreaking insights into viral behavior and immunity. They exemplify the pioneering spirit of scientific exploration, pushing boundaries to reveal the intricate details of viral replication, host range, and neutralization.

However, their work also served as early harbingers of the ethical and safety dilemmas inherent in GoF research.

As we delve deeper into these seminal papers, we will trace their historical context, illuminate their significant findings, and explore how they foreshadowed the necessary precautions and regulatory measures we now associate with gain-of-function research. By journeying back to these early ventures into what would eventually become GoF research, we aim to gain a deeper understanding of this field's evolution and appreciate the careful balance required in pursuing such potentially dangerous yet immensely informative lines of scientific inquiry.

Gain-of-function research, in its essence, refers to the process of deliberately altering an organism's genetic makeup to confer new or enhanced functionalities. In virology, these functionalities often relate to the pathogen's transmissibility, host range, or virulence. While the term "gain-of-function" is relatively recent, the fundamental principles underpinning this type of research have a much longer history, which is instrumental in comprehending the field's current state and regulatory landscape.

The advent of genetic engineering in the latter half of the 20th century opened doors to novel scientific avenues, allowing scientists to manipulate genetic sequences of organisms to understand their function better. In the 1970s and 1980s, as these techniques became more refined, researchers began to experiment with pathogenic organisms, including bacteria and viruses. The objective was straightforward - to gain insights into these pathogens' biology to develop more effective vaccines and therapeutics.

However, the potential implications of this line of inquiry soon raised concerns. In essence, researchers were enhancing the

capabilities of these organisms, potentially making them more infectious or deadly. As such, a seminal moment in the history of GoF research was the Asilomar Conference in 1975. The conference brought together a global assembly of scientists to address the ethical, safety, and biosecurity implications of recombinant DNA technology. The guidelines produced from this conference formed the foundation for subsequent regulations on genetic manipulation of organisms, including gain-of-function research.

In the years following Asilomar, the scientific community gradually began to explore the potential of gain-of-function research in greater depth, leading to important advancements and discoveries, including the works of Rottier and Chow. However, these investigations further underscored the precarious balance between scientific discovery and potential risk.

The influenza research of the early 21st century, specifically the reconstruction of the 1918 Spanish flu and H5N1 avian influenza transmission studies, stirred significant public debate and prompted a reassessment of oversight and regulation. In response, the U.S. government announced a "Pause" on certain gain-of-function studies involving influenza, SARS, and MERS viruses in 2014, followed by the development of a new policy for the review of gain-of-function research in 2017.

Looking back, understanding the historical evolution of gain-of-function research provides a critical perspective. It helps us appreciate how scientific advancements, driven by the need to better understand pathogenic organisms, have walked hand-in-hand with the development of regulations and safety measures. This historical context underscores the importance of ongoing vigilance and active discussion around the potential risks and rewards of gain-of-function research. The lessons learned from these early endeavors continue to shape our approach to such

studies, laying the groundwork for a safer, more ethical pursuit of knowledge in this vital scientific domain.

In the realm of early gain-of-function studies, the work of Rottier and colleagues, published in 2000, stands out for its exploration of the host range of coronaviruses. The paper, titled "Retargeting of coronavirus by substitution of the spike glycoprotein ectodomain: crossing the host cell species barrier," delves into the fundamental mechanisms by which coronaviruses interact with different host species.

Rottier's research aimed to manipulate a critical structural component of coronaviruses - the spike glycoprotein, responsible for the virus's entry into host cells. Using the principles of gain-of-function research, Rottier and his team utilized targeted RNA recombination to construct a chimeric virus. They replaced the spike glycoprotein of the mouse hepatitis virus (MHV) with the ectodomain of the spike protein from the feline infectious peritonitis virus (FIPV).

This bold experiment yielded profound results. The manipulated virus, known as fMHV, displayed a complete switch in species specificity. It gained the ability to infect feline cells, all the while losing its capacity to infect murine cells in tissue culture. This observation was groundbreaking, offering tangible evidence that the host cell range of coronaviruses is primarily dictated by the interaction between the spike protein and the virus receptor on the host cell.

At the time of its publication, this study provided the scientific community with invaluable insights into the host range of coronaviruses and their ability to cross species barriers. More than simply proving a theoretical concept, Rottier's study showed that such manipulation was possible in the laboratory, something

that had not been achieved to such an extent before. The practical implications of these findings were substantial, signaling that similar methods could potentially be employed to study other viruses, and underlining the necessity for stringent safety precautions.

This work also underscored the possible risks associated with such experiments. As the paper itself highlights, the creation of a chimeric virus that can cross species barriers also implies the potential for unintended consequences, such as the emergence of new diseases. It is noteworthy that these concerns and implications predate the contemporary debates about gain-of-function research, emphasizing the foresight and scientific responsibility of the researchers involved.

In retrospect, the experiment conducted by Rottier and colleagues was an early foray into the territory of gain-of-function research that later became the subject of intense scrutiny and regulation. This pioneering work underscored the potential of targeted genetic manipulation to both advance our understanding of viral behavior and pose risks that require careful management. As such, the study stands as an important chapter in the history of gain-of-function research, marking the beginnings of a scientific journey that continues to shape our response to emerging infectious diseases.

To truly appreciate the gravity of Rottier's early gain-of-function research, it's vital to delve into the intricacies of the experimental procedures and understand how this groundbreaking study was conducted. The study revolved around two coronaviruses: the Mouse Hepatitis Virus (MHV) and the Feline Infectious Peritonitis Virus (FIPV).

MHV primarily affects mice, and FIPV infects cats, with each virus demonstrating a high degree of host specificity. The researchers hypothesized that the spike glycoprotein (S protein), a structure protruding from the surface of coronaviruses, played a central role in determining this host range. In other words, the particular structure of the S protein allowed MHV to infect mice but not cats, and vice versa for FIPV.

To test this hypothesis, Rottier's team embarked on a bold experiment: They decided to replace the S protein ectodomain of MHV with that of FIPV using targeted RNA recombination. This novel technique involves swapping specific portions of the viral genome to create a "chimeric" virus that carries genetic material from two different viruses.

After constructing the chimeric virus, which they designated fMHV, they assessed its ability to infect both feline and murine cells in tissue culture. Remarkably, they found that fMHV had acquired the ability to infect feline cells, which regular MHV could not do, and simultaneously lost the capacity to infect murine cells.

This clear-cut switch in host range strongly supported the hypothesis that the coronavirus host cell range is determined predominantly by the interactions between the S protein and the virus receptor on the host cell. The study essentially demonstrated that modifying a particular region of the coronavirus could effectively change the host species that the virus can infect, a significant breakthrough at the time.

But the implications of this work went beyond merely affirming a theoretical postulate. This study set a precedent, establishing that it was indeed possible to manipulate the host range of a virus in the laboratory. This was a crucial development in the context of gain-of-function research, as it offered a method to explore the

viral behavior and adaptations in different host species, informing vaccine development and therapeutics.

However, the significance of this study was not only limited to its potential benefits. The successful creation of a chimeric virus also highlighted the inherent risks associated with gain-of-function studies. The possibility of creating viruses with altered host ranges brought forth serious considerations about potential biosafety and biosecurity issues, pointing to the need for stringent regulation and oversight in such research endeavors. Hence, the Mouse Hepatitis Virus and Feline Infectious Peritonitis Virus study stands as a pivotal instance in the evolution of gain-of-function research, illustrating both its immense potential and its intricate challenges.

In addition to Rottier's exploration into host species specificity, early gain-of-function research also ventured into understanding the mechanisms of virus neutralization and immune evasion. A particularly notable instance of this is the work of Chow and colleagues in 1991, involving the poliovirus. Titled "Identification of residues in VP2 that contribute to poliovirus neutralization antigenic site 3B," this work marked one of the first attempts to manipulate the virus's neutralization sites, with the aim of understanding their structure and function in greater depth.

Poliovirus is a human pathogen infamous for its paralyzing effects, particularly in children. As with other viruses, certain regions on its surface, known as neutralization sites, are recognized by antibodies, which subsequently bind to these sites and neutralize the virus, preventing it from infecting cells. For poliovirus, these sites are present on its capsid proteins, including VP2. By understanding these sites, scientists hope to devise better vaccines and antiviral treatments.

Chow's team focused on a specific neutralization site, 3B, on the VP2 protein of the poliovirus. They utilized site-specific mutagenesis methods to introduce amino acid substitutions at specific residues within this site, creating versions of the virus that were structurally different at a molecular level. It was a pioneering venture at the time, employing innovative genetic techniques to manipulate a pathogen to understand its interaction with the host's immune system.

The results of this study shed light on the fact that even small changes to the virus's neutralization sites could impact its ability to evade the immune response. Many of the amino acid substitutions resulted in viruses that displayed partial or complete resistance to neutralization by antibodies that targeted site 3B. This indicated that even minor mutations in these regions could potentially lead to immune escape variants of the virus.

However, the study also found that many of these substitutions resulted in nonviable viruses or ones with compromised growth characteristics. This suggested a delicate balance between the virus's ability to mutate for survival and the functional constraints it faced to remain viable.

This research by Chow and colleagues was indeed groundbreaking. It provided an early glimpse into the implications of manipulating the neutralization sites of viruses, underscoring the potential for both advancing our understanding of viral behavior and the risks associated with such experiments. As with Rottier's study, the early work of Chow on poliovirus signified a crucial juncture in the historical trajectory of gain-of-function research, offering novel insights while simultaneously bringing to light the need for a cautious approach in conducting such studies.

In order to delve into the intricacies of the groundbreaking experiments conducted by Chow and his team on poliovirus and fully understand their implications at the time, we need to delve deep into the essence of their work. This line of research explored the delicate dance between virus and host, focusing specifically on the interface where the two meet: the viral neutralization sites.

Poliovirus, a non-enveloped RNA virus that has historically caused debilitating disease in humans, served as their model organism. The virus's outer shell, or capsid, is composed of proteins VP1, VP2, VP3, and VP4, with VP1, VP2, and VP3 being exposed on the virus surface and containing the sites that antibodies bind to for neutralization. The specific focus of Chow's work was on a region in VP2 known as neutralization antigenic site 3B.

Their approach involved site-specific mutagenesis, a then-novel technique allowing for precise genetic manipulation at the molecular level. They targeted specific amino acid residues in VP2 that were suspected to be part of the site 3B region. By creating versions of the poliovirus with different amino acid substitutions at these residues, they essentially manufactured a variety of structural variants of the virus.

This detailed manipulation allowed the team to map the involvement of these residues in the formation of the neutralization site 3B. Their results confirmed that the residues they targeted indeed contributed to forming this site, and they also identified a new loop region within site 3B.

One striking finding from this study was the observation that many of the amino acid substitutions led to nonviable virus mutants or mutants with impaired growth characteristics. This suggested that the targeted residues and their surrounding regions had roles beyond merely serving as targets for neutralizing antibodies. They

might also be crucial for the virus's structural integrity or its ability to replicate.

Chow's research findings, taken together, demonstrated the dual-edged sword of viral mutation: on one hand, changes to the neutralization sites could confer resistance to antibodies, potentially leading to vaccine escape. On the other hand, some changes might render the virus nonviable, indicating structural or functional constraints on viral mutation.

Overall, Chow's study was a groundbreaking exploration into the manipulation of viral neutralization sites. This research provided key insights into how viruses might escape immune detection, which would be crucial for future vaccine design and development. It also underlined the potential risks and unknowns involved in gain-of-function research, emphasizing the need for careful scientific rigor and strict oversight in such studies. With this, Chow's work further highlighted the complex interplay between virus and host, shaping our understanding of viral pathogenesis and immunity.

The early gain-of-function studies conducted by Rottier and Chow were truly transformative, fundamentally altering our understanding of viruses and their interactions with hosts, and offering important implications for vaccine development and antiviral therapeutics.

Starting with Rottier's work, the manipulation of the coronavirus spike protein and the ability to switch host cell species specificity suggested that vaccines could potentially be tailored to target specific species or even particular strains of viruses. By understanding the molecular interactions between the virus spike protein and its receptor, vaccine developers could design more precise interventions to block these interactions, preventing viral

entry into cells and thus thwarting infection. Additionally, this understanding could be used to potentially predict which species could be susceptible to a particular virus, aiding in the proactive development of vaccines or treatments.

Similarly, Chow's work also significantly informed vaccine development, particularly in understanding the mechanisms of viral immune evasion. The fact that minor changes in viral neutralization sites could lead to significant resistance against neutralizing antibodies underscored the necessity of considering viral mutations during vaccine development. Vaccines often work by stimulating the immune system to produce neutralizing antibodies against these specific sites. If the virus mutates at these sites, the efficacy of the vaccine can be compromised.

This realization emphasized the need for vaccines that could elicit broad, robust immune responses, not just against one specific site, but multiple regions of the virus. It also underlined the importance of surveillance and the development of next-generation vaccines that could quickly be adjusted in response to new viral variants.

On the therapeutics side, understanding the critical residues and structures involved in viral replication and host-cell entry could guide the development of antiviral drugs. If a residue or a protein is found to be crucial for the virus's ability to infect and replicate in host cells, then drugs could be designed to target and inhibit these key components. For instance, antiviral drugs could be designed to block the interactions between the coronavirus spike protein and its receptor, similar to the mechanisms of action for some current COVID-19 therapeutics.

Overall, these early gain-of-function studies played a foundational role in paving the way for modern vaccine and antiviral drug development. They provided a deeper understanding of viral

biology and pathogenesis, which is crucial for designing effective interventions to combat viral diseases. However, they also highlighted the inherent risks associated with such research, particularly regarding the potential for unintended consequences, which continue to inform the ongoing discourse around the ethics and regulation of gain-of-function research.

The early gain-of-function (GoF) studies by Rottier and Chow, while highly instructive, also underscored the potential risks inherent in this kind of research. The capacity to manipulate a virus's host range, or to induce changes in its key neutralization sites, highlighted the potential for unintended and possibly catastrophic consequences. It was this dual-edged nature of GoF research that led to a significant turning point in its regulation: the NIH (National Institutes of Health) pause on GoF research in 2014.

This pause on funding and conducting certain GoF research involving influenza, MERS, and SARS viruses was implemented due to rising concerns about the potential risks associated with such research. The ability to make pathogens more potent or to extend their host range could have serious biosafety and biosecurity implications if such altered pathogens were accidentally or intentionally released.

The research done by Rottier and Chow arguably laid the foundation for such concerns. Rottier's successful switching of host specificity in coronaviruses demonstrated the potential for enabling a virus to jump species barriers artificially, a possibility with profound implications for zoonotic spillover events. Similarly, Chow's research on poliovirus neutralization sites illustrated how genetic manipulations could potentially create viruses resistant to existing vaccines or treatments.

While these early experiments were conducted with the goal of advancing scientific knowledge and weren't themselves a cause for alarm, they were prescient in demonstrating the possibilities of GoF research. These studies, along with others, undoubtedly influenced the discussions leading up to the NIH pause.

During the pause, the NIH commissioned a thorough risk and benefit assessment of GoF research, aiming to create a more robust and transparent system of oversight. This resulted in the release of a framework in 2017, designed to guide the funding decisions on proposed research involving potential pandemic pathogens.

The NIH's 2014 pause, therefore, served as an acknowledgment of the power and potential risks of GoF research. It represented a commitment by the scientific community and policymakers to proceed cautiously, taking both the potential benefits for public health and the possible risks into account. The pause emphasized the critical importance of rigorous biosafety and biosecurity measures, as well as the need for transparency and public dialogue in this domain of research. It was a pivotal moment, shaped in part by the early experiments of Rottier and Chow, marking the maturation of the regulatory landscape surrounding gain-of-function research.

The early gain-of-function (GoF) experiments conducted by researchers like Rottier and Chow, while providing invaluable insights into viral biology and pathogenesis, also underscored the ethical dilemmas intrinsic to this type of research. The very capability that makes GoF research a powerful tool for understanding disease dynamics and developing therapies also gives it the potential to generate organisms with enhanced pathogenic potential. The ethical conundrum lies in balancing the potential benefits against the possible risks.

Ethically, the foremost concern with GoF research is the principle of "do no harm." This principle obligates researchers and medical professionals to avoid actions that could potentially harm individuals or society. By making pathogens more virulent or increasing their host range, GoF research runs the risk of accidental release of these potentially dangerous organisms, leading to outbreaks or pandemics.

Furthermore, GoF research also holds the potential for misuse. Enhanced pathogens could, in theory, be employed for bioterrorism. While such a risk might be low, the potential impact could be high, thus necessitating serious ethical consideration.

In response to these ethical dilemmas and the broader public concerns, regulations and oversight of GoF research have evolved over time. The early studies by Rottier and Chow would have influenced this development by showcasing both the immense potential and risks of this research field.

The NIH's 2014 pause on GoF research marked a significant step in this evolution. This pause was not just about halting certain types of GoF research temporarily; it was also a commitment to thoroughly examine the risk-benefit ratio of such research and to develop a robust framework for assessing and overseeing GoF studies in the future.

This led to the 2017 framework that provides guidance on funding decisions for proposed research that might create, transfer, or use potential pandemic pathogens. This framework incorporates several factors, including the scientific merit of the research, the potential public health benefits, the biosafety and biosecurity measures in place, and the potential risks associated with the research.

It's important to note that ethical and regulatory evolution is ongoing. As our scientific capabilities advance and as we

continue to learn from experiences such as the COVID-19 pandemic, the ethical debates and regulatory decisions around GoF research will continue to evolve. Thus, it's crucial to maintain an open, transparent dialogue among scientists, ethicists, policymakers, and the public to navigate this complex and dynamic landscape.

Early gain-of-function (GoF) experiments such as those conducted by Rottier and Chow offer a wealth of lessons regarding the potential risks and benefits of this type of research. These pioneering investigations, though conducted decades ago, are highly relevant to the scientific community even today, especially in our current era marked by both remarkable advancements in genetic engineering and an increasing incidence of emerging infectious diseases.

One critical lesson from these early studies is the dual nature of GoF research. On one hand, such studies provide invaluable insights into viral biology, host range, and immune evasion tactics. For instance, Rottier's experiments on the switching of host specificity in coronaviruses significantly enhanced our understanding of how these viruses can cross species barriers. Similarly, Chow's work on mutating poliovirus neutralization sites shed light on how viruses could potentially escape neutralization by antibodies, a vital aspect of vaccine design and effectiveness.

These experiments underscore the fact that GoF research can be an incredibly powerful tool for understanding disease dynamics, informing public health strategies, and advancing therapeutic developments. This knowledge is particularly critical when facing public health emergencies caused by novel pathogens.

On the other hand, these studies also highlight the potential risks associated with GoF research. The very manipulations that make

this research so informative can also render pathogens more virulent or broaden their host range, potentially leading to the creation of organisms with enhanced pandemic potential. This aspect of GoF research underscores the importance of stringent biosafety and biosecurity measures to prevent accidental or intentional release of enhanced pathogens.

The lessons gleaned from these early experiments are particularly relevant for guiding future research. They underscore the need for a rigorous risk-benefit analysis before undertaking GoF studies, careful experimental design to minimize potential risks, stringent laboratory safety protocols, and transparent communication of research goals, methodologies, and findings to the scientific community and the public.

Moreover, these lessons emphasize the importance of regulatory oversight in GoF research. As our technological capabilities continue to advance, regulatory frameworks need to be continually assessed and updated to ensure they are keeping pace with scientific developments and adequately addressing potential risks.

In essence, the early GoF experiments by Rottier and Chow serve as a reminder of the careful balance that needs to be struck in scientific research. While we strive to push the boundaries of our knowledge and capabilities, we must also ensure that we are doing so responsibly and safely, with a keen awareness of the potential risks as well as benefits.

As we move forward in the realm of gain-of-function (GoF) research, the lessons drawn from the past, like those provided by the pioneering works of Rottier and Chow, will continue to be of utmost importance. They serve as the foundation upon which we

can safely and effectively build the future of this highly potent but potentially risky field of research.

A critical part of this forward journey is the continuous reassessment and improvement of regulations and oversight mechanisms that govern GoF research. The 2014 NIH pause on GoF research and subsequent 2017 framework have already set an important precedent in this regard. However, these measures should not be static; instead, they should be dynamic and adaptable, capable of evolving with the scientific landscape and societal expectations. As our technological capabilities continue to advance and we gain more knowledge about infectious diseases, our risk-benefit analyses, safety protocols, and regulatory frameworks will need to be updated accordingly.

Transparency and communication will also be crucial as we move forward. Clear, open dialogues between scientists, policymakers, ethicists, and the public can help ensure that the potential benefits and risks of GoF research are understood by all stakeholders. This, in turn, can lead to more informed decision-making, increased public trust, and a more robust response to public health emergencies.

As for the future directions of GoF research, one can expect that it will continue to be a crucial tool in our efforts to understand and combat infectious diseases. Given the ongoing threat of emerging pathogens and the potential for future pandemics, studies aimed at understanding how viruses can jump species barriers, evade immune responses, and adapt to new environments will be vital.

However, in the light of historical lessons, the scientific community must approach this research with a healthy balance of ambition and caution. It's essential that every GoF study undergo a rigorous risk-benefit analysis before it is undertaken and that all necessary precautions are in place to prevent accidental or

intentional release of enhanced pathogens. Scientists must also be prepared to engage with the public and communicate their research in a transparent and understandable manner, addressing concerns and dispelling misconceptions where necessary.

Lastly, there should be continued efforts to explore and develop alternatives to GoF studies where possible. For instance, computational modeling and in silico experimentation might offer safer ways to study certain aspects of viral behavior and evolution.

In essence, the future of GoF research lies in continuing the delicate balance between its immense potential to advance knowledge and public health, and the need for responsible stewardship to mitigate potential risks. It's a path that will require constant vigilance, open dialogue, and a commitment to ethical and safe scientific practice.

In conclusion, the early gain-of-function (GoF) experiments by Rottier and Chow, conducted decades ago, continue to hold significant relevance and lessons for the contemporary scientific landscape. These pioneering studies not only advanced our understanding of viral biology, but also laid the groundwork for the regulatory frameworks and ethical considerations that guide today's GoF research.

Rottier's study provided invaluable insights into how coronaviruses can cross species barriers by manipulating the spike glycoprotein's ectodomain, a finding with profound implications for our understanding of zoonotic diseases and the development of future vaccines. Similarly, Chow's research on the mutability of poliovirus neutralization sites broadened our knowledge of how viruses can potentially escape neutralization by

antibodies, offering critical insights for vaccine design and antiviral strategies.

However, these early GoF experiments also brought to light the potential risks associated with this kind of research. The manipulation of viral properties to enhance infectivity or broaden host range underscored the dual-use nature of GoF research and highlighted the urgent need for stringent biosafety and biosecurity measures.

The lessons derived from these early experiments have played a crucial role in shaping the subsequent regulatory and ethical landscape of GoF research, including the establishment of the NIH Pause on GoF Research in 2014. They underscore the importance of a rigorous risk-benefit analysis before undertaking such studies, stringent laboratory safety protocols, and the need for transparent communication with the scientific community and the public.

As we navigate the future of GoF research, the insights gained from these early studies remain critically important. They remind us of the careful balance that needs to be struck between advancing scientific knowledge and safeguarding public health. They also highlight the necessity for continuous reassessment and improvement of regulatory and oversight mechanisms in line with scientific and technological advancements.

The enduring significance of these early GoF experiments underscores the importance of revisiting and reevaluating past research. It reminds us that, while our scientific understanding and technological capabilities may evolve, the lessons from the past continue to provide valuable guidance for the future. In this way, the pioneering works of researchers like Rottier and Chow continue to reverberate through the corridors of scientific

research, informing, guiding, and cautioning us as we continue our quest for knowledge and the betterment of human health.

References

Identification of residues in VP2 that contribute to poliovirus neutralization antigenic site 3B (1991) C Reynolds, G Page, H Zhou, M Chow. Virology 184:391-396

Retargeting of coronavirus by substitution of the spike glycoprotein ectodomain: crossing the host cell species barrier (2000) L Kuo, GJ Godeke, MJ Raamsman, PS Masters, PJ Rottier. J Virol. 74(3):1393-1406